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Glucocorticoid receptors function in the pathophysiology of brain hypoxia

Receptory glikokortykosteroidów – rola w patofizjologii niedotlenienia mózgu

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Summary

Glucocorticoid receptors are ligand-activated transcription factors, which play an important role in the brain, mainly in stress response regulation. There are two types of receptors for glucocorticosteroids: mineralocorticoid receptors (MR) with high-affinity for the ligands and glucocorticoid receptors (GR) with a tenfold lower affinity. Selective activation of the receptors during hypoxia may decide neuronal fate, especially in the hippocampus. Depending on the severity of hypoxia-induced damage, neurons undergo necrosis or apoptosis. In the penumbral region, where neurons die mainly through the process of apoptosis, selective GR activation increases excitotoxicity, interferes with apoptotic signalling pathways and causes energy deficit in the cells, all of which promote cell death. On the other hand, selective MR activation seems to be neuroprotective. It is suggested that the main role of MR in neuroprotection is to regulate the balance between anti- and proapoptotic proteins from bcl-2 family.

Keywords: glucocorticoid receptors • mineralocorticoid receptors • hypoxia • neuroprotection • apoptosis

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Abbreviations: **ACTH** – adrenocorticotrophic hormone; **ADX** – adrenalectomy; **AF** – transactivation region; **BDNF** – brain-derived neurotrophic factor; **CRH** – corticotropin-releasing hormone; **DEX** – dexamethasone; **DG** – dentate gyrus; **GR** – glucocorticoid receptor; **HIF-1** – hypoxia induced factor; **HPA** – hypothalamo-pituitary-adrenal axis; **HRE** – hormone response element; **HSD11β** – 11β hydroxysteroid dehydrogenase; **HSP** – heat shock protein; **MR** – mineralocorticoid receptor; **LBD** – ligand binding domain; **LTP** – long-term potentiation; **NR** – nuclear receptor; **TNF** – tumour necrosis factor.

INTRODUCTION

Glucocorticoid receptors are the receptors for steroid hormones synthesized in the adrenal cortex: glucocorticosteroids (glucocorticoid receptors, GR or, according to other nomenclature, NR3C1) and mineralocorticoids (mineralocorticoid receptor, MR; NR3C2). Depending on the concentrations of the common of these receptors (cortisol in humans and corticosterone in rodents) different processes occur in neuronal cells, especially those of the hippocampus. MR activation improves neuronal survival and stimulates proliferation, whereas the activation of GR results in increased neurodegenerative processes [42, 65, 70].

The brain is particularly vulnerable to homeostatic disturbances, including oxygen deficiency. In the core hypoxic zone, neurons die mainly via uncontrolled necrotic death, while in the, so called, penumbral area many cells die through apoptosis, which is a highly controlled process. After hypoxia, the damage to the tissues is mainly concentrated in the hippocampus, which has far-reaching consequences for the whole organism, due to the pivotal role of this structure in the stress response modulation or in the formation of memory traces. Anoxia/ischemia in the hippocampus upregulates the expression of both types of glucocorticoid receptors MR and GR [31, 45]. They regulate cellular response mainly through direct activation or suppression of target genes. However, the final result of MR and GR activity depends heavily on cell's states, which is affected by such signalling substances as neurotransmitters, hormones or cytokines [13]. More importantly, the synthesis or release of these substances, as well as concentrations and affinity of their receptors undergo significant changes in hypoxic conditions [54, 66].

High density of glucocorticoid receptors (GR) seems to be one of the underlying causes of the vulnerability to hypoxia/ischemia. Their activation in hypoxic conditions is an important element of the cascade leading to neuronal death. However, in the penumbral area, the increase in the concentration of MR mRNA has been observed. This may indicate the development of an adaptive neuroprotective response based on MR activity [65]. Hypoxia constitutes a great threat for the life and health of endothermic vertebrates, starting with the fetal period. The pathophysiology of hypoxia is multilayered and conditions leading to hypoxia, mainly as a result of ischaemic and haemorrhagic strokes, vary. In contrast to most diseases, hypoxia is a sudden, unpredictable event, often unnoticeable with delayed damaging effects. The cells which die subsequently to hypoxia in the process of necrosis are impossible to save, but the extended in time (for about one week) apoptotic death provides a hope for significant decrease of the damage.

The article covers the mechanisms underlying the bidirectional activity of glucocorticoid and mineralocorticoid receptors: on one hand, being one of

the main elements of pathological processes during hypoxia (GR) and, on the other hand, promoting neuroprotection (MR).

GLUCOCORTICOID (GR) AND MINERALOCORTICOID (MR) RECEPTORS STRUCTURE

Glucocorticoid receptors belong to the family of nuclear receptors (NR). Nuclear receptors are ligand-dependent transcription factors, which regulate a variety of intracellular processes [46]. 48 NR-coding genes have been identified in the human genome. However, the number of receptor types is greater, because of many splicing-related alternative protein isoforms [64]. Nearly half of the human NR are classified as orphan receptors, whose endogenous ligand have not been identified yet [20].

A two-stage model of NR activity was proposed in 1967: (I) upon hormone binding the receptor is activated and (II) as a transcription factor binds to a specific DNA fragment, regulating genes. Ten years later, genes of 17 beta-estradiol and glucocorticoid receptors were cloned, and they became the first known transcription factors [11].

All the NR, regardless of the kind of ligand they bind, display a homologous structure and comprise of 5 domains: A/B, C, D, E, F [30]. N-terminal A/B domain exhibits the biggest diversity in the NR family, and it contains fragments that bind regulatory proteins responsible for tissue-specific action of receptors. The A/B domain contains AF-1 fragment, which is thought to be a ligand-independent transcriptional activator. AF-1 activating potential is much lower than that of the ligand-dependent AF-2 fragment, which is located in the E domain. The role of N-terminal AF-1 fragment has not been fully elucidated. Structural studies suggest that it may bind additional transcription factors and contribute significantly to allosteric regulation of the receptors [39, 41]. DNA-binding C domain (DBD) and ligand-binding E domain (LBD) are essential for the NR activity and both display a higher level of conservation than the AB domain. In all the NR, C domain has a characteristic DNA-binding structure of zinc fingers. Between C, and E domains, there is a short fragment D, playing the role of an elastic linker. E domain has a ligand-binding site and it recruits transcription coactivators, which makes it the main target of the pharmaceutical research; moreover, in most receptors, E domain is responsible for dimerization [30].

Many nuclear receptors exist in isoforms due to alternative splicing and the presence of alternative translation initiation sites. Understanding the function of specific protein variants is very important in relation to tissue-specific glucocorticoid receptors actions. It is known that in specific tissues different kinds of MR and GR isoforms are expressed. Isoforms may be elements of various pathways, which makes the biology of GR and MR particularly complex.

GR-coding gene, similarly to MR-coding gene, contains 9 exons. As a result of alternative splicing of a precursor mRNA, 5 subtypes of the GR receptor (GR α , GR β , GR γ , GR-A and GR-P) and 3 MR receptor subtypes (MR α , MR β and MR γ) may be synthesized. Isoforms perform different functions. For example, GR β variant is shorter from GR α by the last 15 amino acids and it is unable to bind cortisol. However, it is constitutively expressed in the nucleus and can regulate transcription of specific genes [61, 74]. Additionally, particular isoforms are expressed during various pathological processes. For instance, reduced temperature during ischemia causes the increase of MR β isoform transcription, which promotes neuronal survival [33].

MR and GR receptors form a complex with chaperone proteins HSP (heat shock proteins) family, HSP90 and HSP70. These proteins stabilize the receptors and decrease their affinity to DNA. Upon ligand binding, HSP dissociate from MR/GR, which exposes the DNA-binding domain [26, 35, 59]. Most are localized in the nucleus, however, the inactive MR and GR are present in the cytoplasm. The binding of the ligand and subsequent HSP dissociation exposes nuclear localization signals, which leads to translocation of the receptors to the nucleus [62] (Fig. 1).

ENDOGENOUS LIGANDS OF GLUCOCORTICOID AND MINERALOCORTICOID RECEPTORS

The main endogenous ligands of glucocorticoid receptors are aldosterone (for MR) and glucocorticosteroids: cortisol/corticosterone (for GR and MR). Endogenous glucocorticosteroids (cortisol, corticosterone) have a broad spectrum of action. They are involved in the functioning of almost all cells, and it is estimated that they directly or indirectly regulate the activity of 10% of human genes [7]. The distribution of MR and GR in an organism reflects the physiological role of their ligands [27, 60, 69].

Aldosterone controls the expression of genes involved in the regulation of water and salt balance in the organism. Its major role is to upregulate the renal sodium reabsorption and to induce the release of potassium and hydrogen ions into renal collecting tubules. Furthermore, aldosterone receptors MR are found in organs such as kidneys, sweat glands, salivary glands, and colon. By regulating water balance, MR also affect blood pressure, thus expressed also in the heart cells [25]. MR are also located in the brain, primarily in the hippocampus, where their ligands are glucocorticosteroids. Glucocorticoid receptors GR are located in almost all cells of the

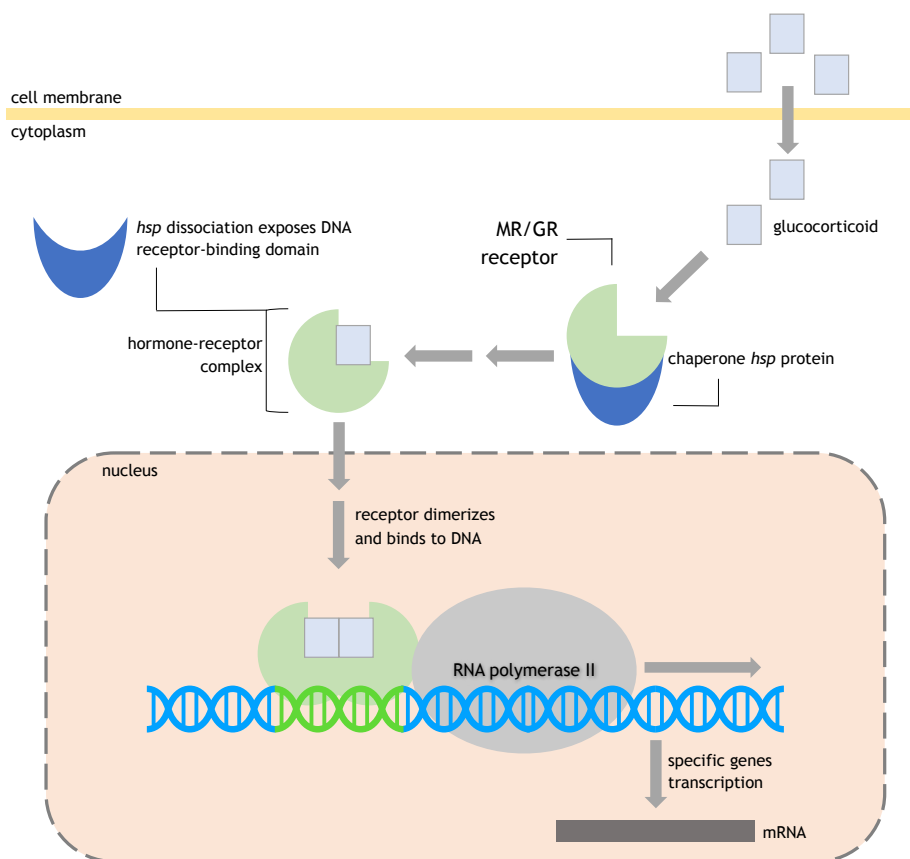


Fig. 1. The structure of glucocorticoid and mineralocorticoid receptors (adapted from [6])

Table 1. Comparison of selected properties of glucocorticoid (GR) and mineralocorticoid (MR) receptors

	GLUCOCORTICOID RECEPTOR	MINERALOCORTICOID RECEPTOR
Ligand	cortisol corticosterone	cortisol corticosterone aldosterone
Antagonist	mifepristone (non-specific) RU-43044	spironolactone RU-28318
Cortisol/corticosterone affinity	low	high
Localization	all cell types particularly high density in the HPA axis structures	kidneys heart sweat glands limbic system: hippocampus

body. Particularly high density of GR is observed in the hypothalamic paraventricular and supraoptic nuclei and in pituitary corticotrophic cells, where they play a central role in the regulation of the hypothalamo-pituitary-adrenal [23, 29, 34].

The main function of glucocorticosteroids is to manage the broadly understood stress response, and their release depends on the activity of the hypothalamo-pituitary-adrenal axis. HPA axis is one of the neuro-hormonal systems associated with the stress response. Corticotropin (CRH), which is synthesized in the paraventricular nucleus of the hypothalamus, stimulates the pituitary gland to secrete the adrenocorticotrophic hormone (ACTH). This hormone stimulates the cells of the adrenal cortical layer to synthesize glucocorticosteroids. The stress stimuli increase the secretion of the glucocorticosteroids into the blood [13, 29, 42, 65].

Corticosterone prevents excessive stimulation of the HPA axis due to negative feedback at the level of the pituitary, hypothalamus and hippocampus [13, 31]. Such regulation of the body's stress response is possible due to the interaction of corticosterone with steroid receptors: mineralocorticoid (MR) and glucocorticoid (GR), which are highly expressed in the hippocampus. The corticosteroid release may largely depend on the activity of mineralocorticoid receptors, whose inhibitory role in modulating the basic activity of the HPA axis [13].

In addition, due to the important role of glucocorticosteroids regulation, high concentrations of GR are found in the liver and adipose tissue. These hormones control the energy metabolism by stimulating the catabolism of carbohydrates and proteins in response to greater

energy demand during stress. They also regulate the immune response, and their role in immunosuppression has been used in pharmacology for many years.

Glucocorticosteroids also play an important role in the development of the central nervous system and in brain plasticity processes in adulthood. Glucocorticosteroid-induced increase in the hippocampal levels of brain-derived neurotrophic factor (BDNF) after exposure to stress seems to be a basic neuroadaptive mechanism that determines adaptation of an organism to new conditions [10, 21].

THE PHYSIOLOGICAL ROLE OF GLUCOCORTICOID AND MINERALOCORTICOID RECEPTORS

Due to the structural similarity of steroids and homologous structures of their receptors, MR and GR do not bind exclusively to one type of ligand. MR have the same affinity for aldosterone as for glucocorticosteroids [22]. Furthermore, contrary to the adopted nomenclature, MR has a 10-fold greater affinity to than GR. Biochemically MR is the primary glucocorticoid receptor, which has significant physiological consequences [42, 65].

Glucocorticosteroids, circulating in the blood at 100 times higher concentration than aldosterone, compete with it for the active site of MR, which are located in the cells of organs responsible for fluid and electrolyte balance. In the cytoplasm of these cells an enzyme 11- β hydroxysteroid dehydrogenase 2 (HSD11 β 2) is present. It inactivates cortisol and corticosterone by oxidizing it to inactive cortisone and 11-deoxycorticosterone respectively. In the absence of active glucocorticosteroids, MR bind aldosterone. Another isoform of this

enzyme (HSD11 β 1) is present in cells responsive to glucocorticosteroids. This enzyme reverses the inactivating reaction and converts cortisone and 11-deoxycorticosterone to, respectively, corticosterone or cortisol [42, 75]. HSD11 β 1 is present in the brain, and particularly high concentrations are observed in the hippocampus, the paraventricular nucleus of the hypothalamus and in the anterior pituitary gland. This suggests that this enzyme affects the level of glucocorticosteroids in the structures important for HPA axis regulation. It has been confirmed by studies on HSD11 β 1 -/- knockout mice, in which abnormal HPA axis activity is observed [28].

The high affinity of MR to glucocorticosteroids makes these receptors activated already at low level of cortisol in the blood. Activation of GR occurs, however, with increased hormone concentration, e.g. before waking up (in a natural circadian rhythm) and during the stress response. In neurons, activation of MR or GR mainly affects transport of ions through the membrane and the levels of produced neurotransmitters. As transcription factors, these receptors primarily trigger a genomic response, although their more rapid, non-genomic action is also observed [42]. This type of action (mainly performed by MR) in response to acute stress manifests itself in the facilitated release of glutamate to the synaptic cleft, changes in post-synaptic potassium currents and in enhancing long-term potentiation (LTP), which is a mechanism important in learning processes [32, 53].

Modulation of the genomic response involving MR and GR is remarkably complex. Based on the analysis of palindromic sequences of promoter elements recognized by these transcription factors (including hormone response element, HRE), it has been concluded that after ligand binding, they dimerize and in this form bind to the corresponding DNA fragment. Studies have shown that in addition to the formation of homodimers (GR-GR), GR may form with MR. Regulation and the role of heterodimerization has not been fully explained, but it has been recently demonstrated that during acute stress, heterodimers bind to different promoters than homodimers, which suggests their important role in the stress response [52, 72]. Furthermore, monomers can regulate the activity of other transcription factors or act repressively by blocking the site for the activating dimer (Figure 2). Some splicing variants of GR and MR, which have no ability to bind the ligand but bind to the specific regulatory of DNA, may work in a similar manner [13, 59].

THE ROLE OF GLUCOCORTICOID RECEPTORS IN HYPOXIA

The neuroprotective and neurodegenerative role of glucocorticosteroids and their receptors becomes particularly significant in neurological crises, such as brain hypoxia/ischemia. During the neurological crisis, the body's natural response is to increase the blood glucocorticosteroid levels. One of the first indications that glucocorticosteroids may potentiate the damaging effects of hypoxia was the observation that glucocorticosteroids administered

after a stroke to prevent inflammation increase brain damage [16]. Similar effects of glucocorticosteroids are also visible in elderly people suffering from atherosclerosis. Atherosclerosis impairs the flow of blood in the brain, which causes its hypoxia. In a stressful situation, e.g. during hospitalization, in people with atherosclerosis an additional release of cortisol results in a rapid impairment of the hippocampus function and related memory disorders and disorientation [47].

To fully understand the role of glucocorticoid receptors in the pathophysiology of hypoxia, it is necessary to outline the changes occurring in neurons under conditions of pathological oxygen depletion. Oxygen or glucose deficiency leads very quickly to the inhibition of ATP resynthesis in cells. The maintenance of resting cell potential depends on the action of ATP-dependent ion pumps. ATP in neurons is mainly used to operate the sodium potassium pumps in order to maintain the resting potential and for repolarization after the action potential. In the absence of ATP, rapid cell depolarization occurs. This is related to the opening of voltage gated ion channels, including calcium channels. An uncontrolled Ca²⁺ influx causes the release of glutamate, the main excitatory neurotransmitter in the central nervous system. Due to the lack of ATP, the reuptake of this amino acid from the synaptic cleft is disturbed. Glutamate activates ionotropic glutamate receptors: NMDA and AMPA, additionally enhancing the influx of Ca²⁺, Na⁺ and Cl⁻ to the cell. Glutamate also binds to metabotropic glutamate receptors, which, by activating phospholipase C, triggers calcium release from intracellular compartments. Simultaneously, along with the Na⁺ and Cl⁻ ions, water flows passively into the cell causing its extensive swelling and inflicting mechanical damage on the cell [17, 54, 57, 66]. Overloading the cell with calcium, which acts as a second messenger, causes the activation of proteolytic enzymes and phospholipases that damage the cell membrane. Furthermore, excessive activation of such enzymes as cyclooxygenase or neuronal nitric oxide synthase causes the formation of reactive oxygen species. If the cell does not undergo a rapid process necrosis, it can enter the intrinsic apoptotic pathway [9].

Apoptosis is programmed cell death – a strictly controlled process aimed at eliminating damaged, defective or super-numerary cells. For this reason, this process is particularly important during development, growth and regeneration, as well as in the body's response to pathological stimuli [18, 58]. Apoptosis is contrasted with necrosis, in which the severity of cellular damage precludes apoptotic reactions. Apoptosis can be distinguished from necrosis based on a microscopic image of a dying cell as well as molecular markers characteristic for these processes. During apoptosis, the integrity of the cell membrane is not disturbed, so that the cytoplasm does not escape into the intercellular milieu. The cell is fragmented into the so-called apoptotic bodies that are subsequently phagocytosed by macrophages. In the case of necrosis, the cell is unable to maintain its membrane potential and proper osmotic pres-

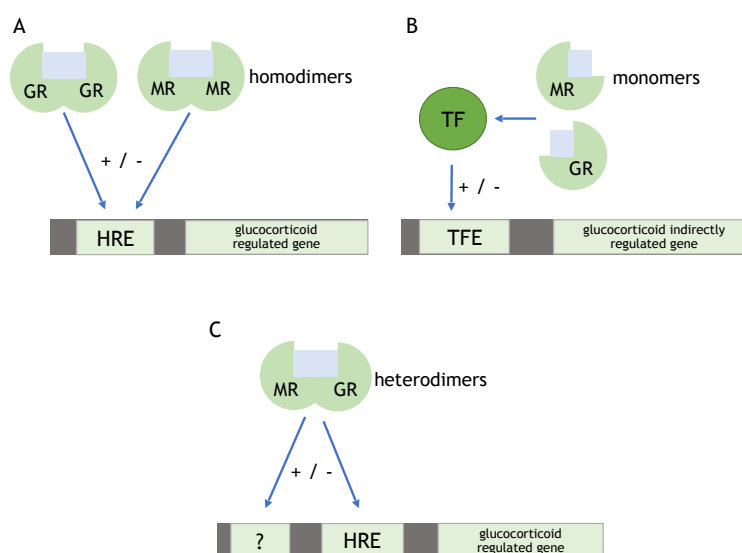


Fig. 2. Mechanisms of regulation of gene expression by glucocorticosteroid receptors: GR and MR; A – typically, the receptors homodimerize after ligand binding. The homodimer binds to the HRE sequence and activates (+) or represses (-) the transcription of a particular gene. B – in the indirect regulation mechanism, ligand-activated monomers modulate the activity of other transcription factors (TFs). C – regulation of transcription through heterodimerization (MR with GR) is the least-studied mechanism. It is suggested that heterodimerization may lead not only to the connection with HRE, but also to previously unknown regulatory sections (adapted from [13])

sure; it swells and breaks, which usually involves damage to the neighbouring cells and the emergence of inflammation. It is believed that there is no distinctive boundary between necrosis and apoptosis, and the observed processes are a continuum, rather than a dichotomy (currently including the necroptosis process, among others) [15, 18].

There are two main apoptotic pathways: extrinsic and intrinsic. The extrinsic one is based on the signalling pathway triggered by the activation of membrane receptors, including TNF (tumor necrosis factor) receptors. Hypoxia-induced neuronal apoptosis is activated mainly by the intrinsic pathway [56]. The intrinsic pathway is controlled by the *bcl-2* family of proteins. Almost all human cells maintain a constant level of these proteins, especially of the proapoptotic: Bax and Bak and antiapoptotic: Bcl-2 and Bcl-X_L. Concentration ratio between the two types of proteins determine the cell fate and is one of the apoptotic markers. Bcl-2 proteins are located in the outer mitochondrial membrane and modulate its permeability. The main element of the apoptotic process is the activation of Bax protein which, by the formation of channels in the outer mitochondrial membrane, causes the release of cytochrome c from the mitochondrial intermembrane space. Bax protein regulates p53 protein, which controls numerous processes in the cell and is a part of signalling pathways associated with DNA repair and apoptosis [55]. Further stages of apoptosis are mediated by proteases from the caspase family [51].

The intrinsic pathway in neurons is similar to that in other types of cells, but the regulation of proteins associated with the apoptotic pathway is more stringent.

Brain development during embryogenesis involves the removal of supernumerary neurons via precisely controlled apoptosis. It is estimated that over half of the neurons are removed in this way during the development. The control of apoptosis must also be maintained in the mature brain. In most brain structures neurogenesis ceases immediately after birth and neurons must perform their function until the end of the individual's life. In consequence, neurons must be equipped with preventive mechanisms to avoid accidental activation of the apoptotic pathway [37, 43].

Necrotic cell death results in the release of cellular contents, including proteolytic enzymes and excitotoxic glutamate, into the extracellular space, which damages neighbouring cells. Cells in the hypoxic core undergo necrosis and this process is very rapid and irreversible. However, around this area, cells maintain integrity. This is the, so-called, penumbral region where neurons can enter the apoptotic pathway. Much depends on the type and duration of hypoxia, the type of neuron, the stage of the cell cycle, the amount of available ATP (indispensable to create a functional apoptosome and to synthesize regulatory proteins) at the time of neurological crisis and the condition of neighbouring cells. In the penumbral area, the process of apoptosis is relatively long-lasting, even up to a week after the episode of hypoxia [44].

The brain area that is particularly vulnerable to hypoxia-induced damage is the hippocampus. The hippocampus is a part of the vertebrate limbic system responsible for memory consolidation and spatial orientation. It forms numerous connections with other brain regions, mainly

with the prefrontal cortex, and also plays a superior role in controlling the activity of HPA axis. Four main anatomical areas are distinguished in the hippocampus: CA1, CA2, CA3 regions comprised of pyramidal neurons and dentate gyrus (DG) consisting of granular neurons. The dentate gyrus is one of the only brain regions where neurogenesis occurs also in mature mammals, i.e. after the development of the central nervous system is complete [14]. Both types of glucocorticosteroid-binding receptors (MR and GR) are highly expressed in the hippocampus, which makes this area of the brain the main target of glucocorticosteroids [13, 65]. Excessive, as well as too low concentrations of glucocorticosteroids increase the vulnerability of the hippocampus to the damage induced by excitotoxicity, oxidative stress or hypoxia/ischemia [71].

ACTIVATION OF GR RECEPTORS AND NEURODEGENERATION

GR are activated when the level of glucocorticosteroids increases, which occurs during the peak of endocrine secretion or after exposure to a stress stimulus [13, 32, 50]. Stress induces the release of stress response hormones regardless of the type of stress factor. Hypoxia/ischaemia is one of such stimuli [67]. The role of enhanced GR activity in the increase of vulnerability of hippocampal cells to excitotoxic or hypoxic/ischemic damage has been confirmed by numerous studies [50, 70, 71]. The administration of specific GR agonists (methylprednisolone and dexamethasone) contributes to the increased damage of nerve cells [2, 73]. Therefore, it is believed that the neurotoxic effects of elevated glucocorticosteroid levels are mediated by glucocorticoid receptors [13], which are activated at high concentrations of these hormones.

The cascade of pathological events under hypoxia begins with an energy crisis which is supposed to be counteracted by the stress hormones and the influx of glucose to the regions of the body that are most mobilized under stress conditions, including the brain. However, with a prolonged (over 30 minutes) exposure to stress hormones, especially to glucocorticosteroids, some cells, such as hippocampal neurons, are affected by a significant fall of glucose level. In the course of hypoxia there is often a synergistic effect of two stimuli: the lack of oxygen and the excessive activation of GR, which cause a decrease in the level of glucose. Exposing hypoxic rats to stress enhances brain damage. This happens when the stress-induced elevated concentration of corticosterone intensifies the energy crisis in neurons [1]. The simultaneous activation of GR and exposure to hypoxia aggravate the brain damage by activating specific pathological processes.

The harmful effects of GR activation under hypoxic conditions are confirmed by studies in which reduction of post-ischemic damage occurs after administration of GR antagonist RU486 or after adrenalectomy [50, 71]. It has also been demonstrated that administration of HSD11 β 1 inhibitors reduces the level of corticosterone, therefore

limits the activation of GR and reduces excitotoxic neuronal damage [63]. Active GR presumably interact with different hypoxia-response genes or with stimuli modulating this response (e.g. HIF-1), for example affecting the distribution of glucose transporters, crucial in the energy crisis (Figure 3) [36, 65]. However, it seems that the neurotoxic action of GR is manifested, above all, in the induction of apoptosis [2], which is why this issue requires a separate discussion.

NEUROPROTECTIVE ROLE OF MINERALOCORTICOID RECEPTORS DURING HYPOXIA

The occurrence of GR in the hippocampus is relatively easy to explain, because it is the primary regulatory centre of HPA axis activity. But what is the reason for the presence of aldosterone receptors (MR) in this area, a hormone that regulates the body's water and mineral balance?

Previous studies have shown that the synthesis and activation of MR during the influence of various types of stressors raise the chance of neuronal survival, suggesting that it presents an adaptive mechanism, activated in response to damage. An increase in the synthesis of MR receptors was found in CA1 region of the hippocampus, where ischemia-induced cell damage occurred [31]. Moreover, it was shown that shifting the balance towards increased MR activity after inhibiting GR receptors protects neurons from damage under ischemic conditions [38]. Elevated MR concentration is also observed in fragments of the hippocampus collected post mortem from people who died due to a cardiac arrest [40].

Animal studies with MR gene deletion or after ADX suggest that MR perform an important function in the proliferation of neurons in the dentate gyrus and also indicate the neuroprotective role of these receptors in CA1-CA4 regions of the hippocampus [45]. Moreover, it was found that concentrations of corticosterone specifically activating MR promote neuronal survival [2]. Staurosporine is a compound used for the experimental induction of apoptosis. However, it occurs that the administration of small doses of staurosporine has a neuroprotective effect. This is most likely associated with MR activation, because blocking these receptors with selective antagonists – spironolactone or RU28318 – inhibits the neuroprotective effect of staurosporine. Increasing number of studies shows a natural rise in MR concentration in response to staurosporine-induced and aging- or hypoxia-related apoptosis of hippocampal cells [33]. Particularly pronounced changes in MR expression are observed in neurons of rats exposed to hypoxia/ischemia under thermal stress conditions (hypothermia and hyperthermia) [33, 45, 68].

In order to develop effective therapies based on the properties of the described receptors, it is crucial to elucidate the mechanism of MR activity. There are no detailed, molecular studies focusing on processes occurring in the hypoxic cell after MR activation, how-

ever, the available data suggest several hypothetical mechanisms. Binding of the ligand to the receptor releases the associated HSP proteins as well as immunophilins. Proteins from the HSP family perform a number of functions related to stress response, including regulation of apoptotic processes [5]. In contrast, immunophilins, which are involved in axonal transport and the formation of synaptic vesicles, prevent protein aggregation, which is one of the basic processes in the course of neurodegeneration [4] (Figure 3). Elevating of the concentration of free forms of chaperone proteins may contribute to neuronal protection, however, it should be borne in mind that the same proteins are associated with GR, the activation of which induces neurodegenerative processes.

Because MR and GR have an adverse effect on cells under specific conditions, it is suggested that the ratio of their concentrations is significant for the survival of the neuron. Increased MR concentration, in response to hypoxia, may indirectly prevent excessive GR activation. It is also indicated that GR and MR expression is dependent on the concentration of active forms of these receptors, e.g. an increase in GR concentration is observed with selective blocking of MR [12].

As mentioned earlier, the effect of glucocorticosteroids via GR enhances the excitotoxicity by over-activation of glutamate receptors. On the contrary, MR are able to inhibit the transcription of subunits of some types of calcium channels and NMDA receptors [32, 65]. The reduction in the number of calcium channels and NMDA receptors activity reduces the influx of calcium ions, which may be crucial reducing the excitotoxic effect implicated by GR activation under hypoxic conditions. However, the most important potential neuroprotective mechanism mediated by MR is their effect on the of anti-apoptotic Bcl-2 and Bcl-X_L proteins [2].

THE ROLE OF GLUCOCORTICOSTEROIDS AND THEIR RECEPTORS IN NEURONAL APOPTOSIS

The most important role of GR and MR in determining neuronal fate after hypoxia/ischemia is their pro- and antiapoptotic effect dependent on the concentration of glucocorticosteroids [32] (Figure 3). The completion of nervous system development is associated with a rapid decline in the anti-apoptotic Bcl-2 protein level, constituting natural end of neurogenesis. Due to the specificity of the dentate gyrus, the concentration of this protein in the granular DG cells remains unaltered, which presumably sustains neurogenesis in this brain region. Simultaneously, stable, relatively low levels of proapoptotic proteins are observed in other areas of the brain [43]. In the dentate gyrus, the ratio of pro- and antiapoptotic protein concentrations determines the cell fate, and therefore experimental studies in which the pro- and antiapoptotic balance is modified allow the role of GR in the apoptosis to be investigated.

Adrenalectomized rats (ADX; surgical removal of the adrenal glands – the natural source of glucocorticosteroids) have changed expression profiles of Bax, Bcl-2, and Bcl-X_L in various areas of the hippocampus after corticosterone administration. In the dentate gyrus (DG), i.e. in the area where neuronal proliferation continues, the concentrations of bcl-2 proteins change after adrenalectomy. Additionally, increased production of Bax is observed, which returns to the baseline level after administration of an exogenous hormone dose, capable of full MR and only partial GR activation. This may indicate that Bax expression or p53 protein activity [8]. However, there are also studies that showed that selective MR blockage does not contribute to the changes in the level of apoptosis-controlling proteins, and it is rather GR that determine the fate of granular cells in DG [45].

The cells of CA1, CA2 and CA3 regions of the hippocampus appear to respond differently to ADX. ADX-induced reduction of corticosterone level causes an increase in anti-apoptotic Bcl-2 protein concentration. The same can be applied to the DG described above, except that in CA areas, especially CA1, there is a marked decrease in Bcl-2 level after corticosterone administration. This may indicate a negative regulation of Bcl-2 expression by glucocorticoid receptors in CA1 region. Simultaneously, it appears that administration of small doses of corticosterone (activating only MR) in drinking water, promotes the expression of Bcl-2 in CA1 and CA3 [8]. However, it should be emphasized that unequivocal conclusions regarding the role of receptors in the survival of hippocampal cells cannot be made only on the basis of studies in which hormone levels were manipulated.

In young (3 month-old) and old (24 month-old) rats, significant differences were demonstrated in the apoptotic coefficient (number of cells undergoing apoptosis per 10.000 intact cells) depending on the activation of specific receptors [2]. To determine the effects caused by a particular receptor, one of the rat groups was given corticosterone at the concentration mainly activating MR, and the other was administered a selective GR ligand – dexamethasone (DEX; synthetic glucocorticosteroid). Administration of corticosterone caused a decrease in the value of apoptotic coefficient in old rats. In contrast, DEX administration caused an increase in this coefficient in both groups, but in older rats, enhanced neurodegeneration due to selective GR activation was observed. Analysis by *in situ* hybridization showed changes in the mRNA level of all bcl-2 family proteins. However, it was the effect of selective MR activation on the level of pro-apoptotic Bax protein that was the most remarkable. MR activation significantly reduced Bax concentration in the dentate gyrus cells, whereas GR activation increased the concentration of Bax proteins. The positive regulation of Bax expression by selective GR activation may also be evidenced by the fact that Bax ^{-/-} mice do not exhibit increased apoptosis after DEX administration. The action of the hormone by MR stimulates the synthesis of Bcl-2 in old rats, which enhances neuroprotection [2].

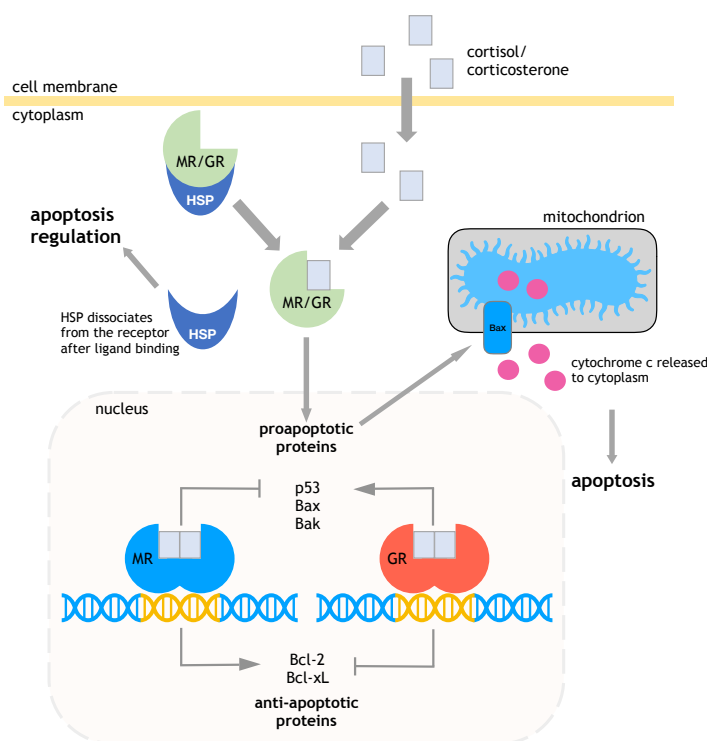


Fig. 3. Scheme of apoptosis regulation through glucocorticosteroid receptors. MR and GR antagonistically activate and inhibit gene expression for proteins regulating the process of apoptosis (arrow with pointed arrowhead and without it mean respectively: positive and negative regulation of the expression)

Moreover, studies have shown a significant increase in the concentration of pro-apoptotic p53 protein after dexamethasone administration, which was not observed in the case of corticosterone administration, suggesting that selective GR activation and increased p53 expression are related [65]. However, it is uncertain whether activation of GR and increased expression of p53 cause apoptosis in all cells, because it has been demonstrated that hippocampal DG neurons do not undergo cell death. Instead, their cell cycle stops in the G1 phase, which means that neuronal proliferation, characteristic this brain region, is inhibited [12]. The involvement of MR in the regulation of p53 expression has also been shown [2, 49]. It is also worth noting that the blockage of MR with spironolactone reduces the level of mRNA for the Bcl-2 protein [49], suggesting that activation of MR receptors determines the expression of anti-apoptotic Bcl-2 protein and may affect the survival of neurons after injury. This phenomenon may be of fundamental importance for inhibiting the delayed neuronal death due to hypoxia/ischemia. Importantly, it seems that the ratio of pro- and antiapoptotic protein concentrations results from different levels of activation of the glucocorticoid receptors being discussed [12].

CONCLUSION

Understanding the complexity of neurodegenerative processes is an enormous challenge for clinicians, but it is essential to develop therapies that are effective at

multiple levels of the cascade of events under hypoxia/ischemia. There are numerous possibilities: inhibition (administration of antagonists) of glutamate receptors, scavenging of free radicals, inhibition of proteins regulating the apoptotic or inhibition of inflammation [19, 54]. Studies on the role of MR and GR in the pathophysiology of hypoxia indicate that they may also be a potential drug target. The presented state of knowledge on the role of glucocorticoid receptors in the pathophysiology of brain damage and their impact on the fate of neurons indicate the scale of the challenge that scientists face. The results of the research reveal the network of interactions within the cell, between cells and the organism and the environment, which should be taken into account when developing new therapies.

Considering the current state of knowledge regarding these receptors, it seems that their activity could both prevent the occurrence of brain damage, as well as eliminate the effects of already existing changes. A research review on the role of MR and GR receptors in the pathophysiology of hypoxia indicates that their activity is of a dose-dependent (hormetic) nature. Hormesis is a bidirectional reaction of an organism to a dose, which stimulates the organism in the range of small doses of the environmental factor and inhibits its vital functions at high doses [48]. Only in the case of a specific (usually low) range of a given parameter (concentration and time of exposure to the hormone)

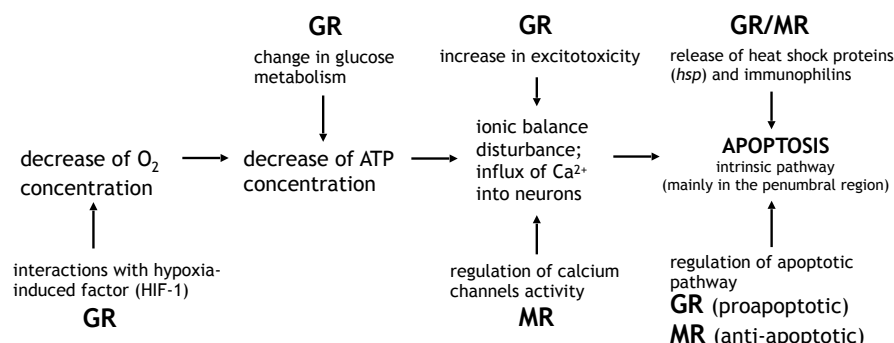


Fig. 4. Summary of the main processes in the pathophysiology of hypoxia in which the regulatory influence of GR and MR receptors was confirmed

an organism is able to maintain homeostasis. Short-term, elevated concentration of glucocorticosteroids is a signal in the physiological norm for an organism and performs an adaptive function. However, chronic exposure to glucocorticosteroids leads to numerous negative consequences [24]. With their enduring elevated level, e.g. in the Cushing's syndrome, when some steroid drugs are administered or when chronic stress occurs, the hippocampus functions are disturbed, mainly due to GR hyperactivity [70]. Small doses of the hormone activate MR that medi-

ate neuroadaptive processes, whereas large doses of corticosterone activate GR and thus, stimulate neurodegeneration [1, 32, 65].

The dichotomous effect of glucocorticoid receptors on the survival of neurons can be applied in the development of new therapeutic strategies. The results of the cited studies indicate the interactions of MR and GR with apoptosis-controlling proteins and thus point to new directions in designing drugs specifically targeted at cells undergoing apoptosis as a result of hypoxia/ischemia.

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